

TABLE 2

	Reference Example 1	Reference Example 2	Reference Example 3	Reference Example 4
Diameter of Liquid pool ( $\mu\text{m}$ )	400	400	400	400
Cell density (cells/ $\text{cm}^2$ )	$8 \times 10^4$	$2 \times 10^5$	$8 \times 10^4$	$2 \times 10^5$
Number of cells (cells)	100	320	100	320
Inner diameter/outer diameter of non-adhesive portion ( $\mu\text{m}$ )	300/600	300/600	300/600	300/600
Diameter of adhesive portion ( $\mu\text{m}$ )	400	400	400	400
Cell arrangement method	IJ	IJ	IJ	IJ
Drying suppression of liquid pool	Oil	Oil	Not used	Not used
Initial arrangement of cells	+	+	+	+
Fixation of cells	+	+	-	-
Maintenance of shape of liquid pool	+	+	-	-

[0239] The present invention includes the following aspects.

[0240] [1] A manufacturing method for a substrate on which nerve cells are arranged, the method including a step of forming one or a plurality of liquid pools by arranging a plurality of liquid droplets containing nerve cells on a substrate using an inkjet method, to form one or a plurality of liquid pools, where substrate has a region in which a cell adhesive material is arranged and a region in which a cell non-adhesive material is arranged; and a step of incubating the liquid pool until the nerve cells to sediment and temporarily adhere onto the substrate to form a cell aggregate, in which the diameter per one liquid pool is  $500 \mu\text{m}$  or less, and the density of nerve cells per one liquid pool is  $10^5$  cells/ $\text{cm}^2$  or more.

[0241] [2] The manufacturing method according to [1], in which one liquid pool contains 7 to 10,000 nerve cells.

[0242] [3] The manufacturing method according to [1] or [2], in which one liquid droplet contains 1 to 50 nerve cells.

[0243] [4] The manufacturing method according to any one of [1] to [3], in which each liquid droplet is arranged to be in contact with the cell adhesive material.

[0244] [5] The manufacturing method according to any one of [1] to [4], further including a step of suppressing evaporation of a liquid in the liquid pool.

[0245] [6] The manufacturing method according to any one of [1] to [5], further including a step of supplying a medium to the substrate on which the cell aggregate is formed.

[0246] [7] The manufacturing method according to [6], further including a step of functionally binding at least two cell aggregates by incubating the substrate to which the medium has been supplied, in which a plurality of the liquid pools are formed in the step of forming the liquid pool, and a plurality of the cell aggregates are formed in the step of incubating.

[0247] [8] The manufacturing method according to any one of [1] to [7], in which the substrate has a region in which the cell non-adhesive material is arranged and a region in which the cell non-adhesive material is not arranged, the region in which the cell non-adhesive material is not arranged has a linear shape, and a width of the linear shape is  $100 \mu\text{m}$  or less.

[0248] [9] The manufacturing method according to any one of [1] to [8], in which the substrate has a porous structure.

[0249] While preferred embodiments of the invention have been described and illustrated above, it should be understood that these are exemplary of the invention and are

not to be considered as limiting. Additions, omissions, substitutions, and other modifications can be made without departing from the scope of the invention. Accordingly, the invention is not to be considered as being limited by the foregoing description and is only limited by the scope of the appended claims.

#### EXPLANATION OF REFERENCES

- [0250] 10 . . . Liquid droplet ejection head
- [0251] 11, 970 . . . Cell ink
- [0252] 12, 25 . . . Liquid chamber
- [0253] 13, 28 . . . Membrane
- [0254] 14, 26 . . . Driving unit
- [0255] 15 . . . Nozzle
- [0256] 16, 27 . . . Vibration application portion
- [0257] 17, 24 . . . Atmospheric opening portion
- [0258] 21, 22, 23 . . . Inkjet head
- [0259] 31 . . . Stage portion
- [0260] 400, 500, 600, 700 . . . Liquid droplet-arranging device
- [0261] 910 . . . Flat plate member
- [0262] 920 . . . Porous member
- [0263] 930 . . . Cell culture carrier
- [0264] 940 . . . First solution
- [0265] 950 . . . Second solution
- [0266] 960 . . . Cell non-adhesive material
- [0267] 980 . . . Cells
- [0268] Pj . . . Ejection waveform
- [0269] Ps . . . Vibration isolation waveform.

1. A manufacturing method for a substrate on which nerve cells are arranged, the method comprising:

a step of forming one or a plurality of liquid pools by arranging a plurality of liquid droplets containing nerve cells on a substrate using an inkjet method, to form one or a plurality of liquid pools, wherein the substrate has a region in which a cell adhesive material is arranged and a region in which a cell non-adhesive material is arranged; and

a step of incubating the liquid pool until the nerve cells in the liquid pool sediment and temporarily adhere onto the substrate to form a cell aggregate,

wherein the diameter per one liquid pool is  $500 \mu\text{m}$  or less, and

the density of nerve cells per one liquid pool is  $10^5$  cells/ $\text{cm}^2$  or more.

2. The manufacturing method according to claim 1, wherein one liquid pool contains 7 to 10,000 nerve cells.